



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460



OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

July 27, 2005

Subject: EPA Id No.: 114004. Amicarbazone: Review of the Developmental Neurotoxicity Study (2001, MRID # 45441301).

TXR # 0050814
DP Barcode No.: D276837
Submission No.: S600000
PC Code: 114004

From: John Doherty *John Doherty 10/29/05*
ReRegistration Branch III
Health Effects Division 7509C

To: Christina Swartz, Branch Chief
and
Kimberly Kosick, Toxicologist
Registration Action Branch II
Health Effects Division 7509C

Through: Catherine Eiden *Cathy Eiden*
Branch Chief
ReRegistration Branch III
Health Effects Division 7509C

The developmental neurotoxicity (DNT) study (2001, MRID # 45441301) with amicarbazone has been reviewed by the *ad hoc* special DNT committee and the study was classified as Acceptable/Non-Guideline. The Non-Guideline status reflects inherent problems with the positive control data base not specific from the testing laboratory rather than the study itself. In addition, the low and mid dose morphometric measurements for the corpus callosum need to be provided to further investigate the statistically significant decrease in this brain structure seen at the high dose in day old in males.

A copy of the DER is attached. The study is further identified and the Executive Summary is in the following table.

NOV 09 2005

Table: Study Reviewed.

Study Reviewed	Executive Summary
<p>870.6300. Developmental neurotoxicity study- rats Bayer (Kansas), Study No.: 109811, March 1, 2001.</p>	<p>In a developmental neurotoxicity study (2001, MRID 45441301), MKH 3586 (97.8-98.4% a.i., batch # 05362/0005) was administered to parent female Wistar rats in the diet at concentrations of 0, 100, 500 or 1000 ppm from gestation day 0 through postnatal day (PND) 21. The average daily intake of MKH 3586 was approximately 0, 8, 39 and 91 mg/kg/day during gestation and about twice that during lactation, for the 0, 100, 500, and 1000 ppm groups, respectively. Special inclusions were that thyroid and neural tissues (10/sex/dose) were assessed histologically on PND 11 and at study termination. <i>Maternal Toxicity.</i> Treatment-related effects were limited to body weight and gain, increased food consumption and decreased food efficiency. During <i>gestation</i>, body weight at 1000 ppm was decreased ($p < 0.05$) of 5% on GD 6, with differences persisting to GD 20 (4%, $p < 0.05$) associated with a 12% decrease ($p < 0.05$) in body weight gain from GD 0 to GD 20. The high dose group was also associated with increased food consumption (13%) and associated decrease in feed efficiency (22%). During <i>lactation</i>, maternal body weight was decreased for high-dose rats; decreases averaged 4% on LD 0 to 9% on LD 14 and LD 21 ($p < 0.05$ or 0.01). Non significantly lower body weights were noted for the 500 ppm dose group of 2-3% but body weight gain during lactation was reduced 28% at 500 ppm and 45% at 1000 ppm. Food consumption during lactation was increased 7% at 500 ppm and 8% at 1000 ppm. The combination of body weight decrease and increase food consumption resulted in a decrease in food efficiency or 33% at 500 ppm and 49% at 1000 ppm. The maternal LOAEL is 500 ppm (39 mg/kg/day) based primarily on decreased feed efficiency (combination of decreased body weight gain and increased feed consumption) during lactation. The maternal NOAEL is 100 ppm (8 mg/kg/day).</p> <p><i>Offspring Toxicity.</i> Treatment had no adverse effects on offspring survival, food consumption, clinical signs, developmental landmarks, FOB, motor activity, auditory startle reflex, learning and memory or brain weight. Decreased body weight and body weight gain were seen in males and females at the mid (500 ppm) and high (1000 ppm) dose groups. At birth, the average body weight of treated offspring was not different from controls at any dose level. By PND 11, body weight was decreased 7-8% ($p < 0.05$) for high-dose male and females, this decrease averaged 11-12% ($p < 0.01$) by weaning on PND 21. Body weight gain was decreased in males and females at the mid (8-13 %; $p < 0.05$ or $p < 0.01$) and high (11-21 %; $p < 0.05$ or $p < 0.01$) dose groups. Offspring in the 500 ppm group had recovered by termination; however, decreased body weight gain persisted to study termination in high-dose animals. On PND 11, the absolute brain weights were decreased in males (12%) and females (7%) at the high dose. -On PND 11, there was a statistically significant ($p \leq 0.01$) decrease (25%) in corpus callosum morphometric measurements of males at the high dose. The offspring LOAEL is 1000 ppm (91 mg/kg/day) based on decreased body weight gain, decreased absolute brain weight, and brain morphometric changes. The offspring NOAEL is 500 ppm (39 mg/kg/day).</p> <p>This study is classified Acceptable/Non Guideline and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) pending receipt and review of the brain morphometric data for the corpus callosum for the low and mid dose groups, and review of the positive control data.</p>

DATA EVALUATION RECORD

MKH 3586 (AMICARBAZONE)

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY - RAT;

OPPTS 870.6300

MRID 45441301

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task No. 02-55

Primary Reviewer:

Cheryl B. Bast, Ph.D., D.A.B.T.

Secondary Reviewers:

Carol Forsyth, Ph.D., D.A.B.T.

Robert H. Ross, M.S. Group Leader

Quality Assurance:

Lee Ann Wilson, M.A.

Signature: Cheryl B. Bast

Date: AUG 26 2004

Signature: Carol Forsyth

Date: AUG 26 2004

Signature: Robert H. Ross

Date: AUG 26 2004

Signature: Lee Ann Wilson

Date: AUG 26 2004

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory is managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

MKH 3586 (Amicarbazone)/114004

OPPTS 870.6300/ OECD 426

EPA Reviewer: John Doherty, Ph.D.
ReRegistration Action Branch 3, Health Effects Division (7509C)
EPA Work Assignment Manager: G. Dannan, Ph.D.
Toxicology Branch, Health Effects Division (7509C)

Signature: [Signature]
Date: 7/27/05
Signature: [Signature]
Date: 7/27/05
Template Version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 114004

DP BARCODE: D276837

TXR#: 0050814

SUBMISSION NO.: S600000

TEST MATERIAL (PURITY): Technical Grade MKH 3586 (97.8-98.4%)

SYNONYMS: Amicarbazone

CITATION: Sheets, L. P. (2001) A Developmental neurotoxicity screening study with technical grade MKH 3586 in Wistar rats. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf Ave., Stilwell, Kansas, 66085-9104. Laboratory report number 109811; March 1, 2001. MRID 45441301. Unpublished

SPONSOR: Bayer Corporation, Agriculture Division, Box 4913, Hawthorne Road, Kansas City, Missouri 64120-0013.

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (2001, MRID 45441301), MKH 3586 (97.8-98.4% a.i., batch # 05362/0005) was administered to parent female Wistar rats in the diet at concentrations of 0, 100, 500 or 1000 ppm from gestation day 0 through postnatal day (PND) 21. The average daily intake of MKH 3586 was approximately 0, 8, 39 and 91 mg/kg/day during gestation and about twice that during lactation, for the 0, 100, 500, and 1000 ppm groups, respectively. Special inclusions were that thyroid and neural tissues (10/sex/dose) were assessed histologically on PND 11 and at study termination.

Maternal Toxicity. Treatment-related effects were limited to body weight and gain, increased food consumption and decreased food efficiency. During *gestation*, body weight at 1000 ppm was decreased ($p < 0.05$) of 5% on GD 6, with differences persisting to GD 20 (4%, $p < 0.05$) associated with a 12% decrease ($p < 0.05$) in body weight gain from GD 0 to GD 20. The high dose group was also associated with increased food consumption (13%) and associated decrease in feed efficiency (22%). During *lactation*, maternal body weight was decreased for high-dose rats; decreases averaged 4% on LD 0 to 9% on LD 14 and LD 21 ($p < 0.05$ or 0.01). Non significantly lower body weights were noted for the 500 ppm dose group of 2-3% but body weight gain during lactation was reduced 28% at 500 ppm and 45% at 1000 ppm. Food consumption during lactation was increased 7% at 500 ppm and 8% at 1000 ppm. The

combination of body weight decrease and increase food consumption resulted in a decrease in food efficiency or 33% at 500 ppm and 49% at 1000 ppm. **The maternal LOAEL is 500 ppm (39 mg/kg/day) based primarily on decreased feed efficiency (combination of decreased body weight gain and increased feed consumption) during lactation. The maternal NOAEL is 100 ppm (8 mg/kg/day).**

Offspring Toxicity. Treatment had no adverse effects on offspring survival, food consumption, clinical signs, developmental landmarks, FOB, motor activity, auditory startle reflex, learning and memory or brain weight. Decreased body weight and body weight gain were seen in males and females at the mid (500 ppm) and high (1000 ppm) dose groups. At birth, the average body weight of treated offspring was not different from controls at any dose level. By PND 11, body weight was decreased 7-8% ($p < 0.05$) for high-dose male and females, this decrease averaged 11-12% ($p < 0.01$) by weaning on PND 21. Body weight gain was decreased in males and females at the mid (8-13 %; $p < 0.05$ or $p < 0.01$) and high (11-21 %; $p < 0.05$ or $p < 0.01$) dose groups. Offspring in the 500 ppm group had recovered by termination; however, decreased body weight gain persisted to study termination in high-dose animals. On PND 11, the absolute brain weights were decreased in males (12%) and females (7%) at the high dose. -On PND 11, there was a statistically significant ($p \leq 0.01$) decrease (25%) in corpus callosum morphometric measurements of males at the high dose. **The offspring LOAEL is 1000 ppm (91 mg/kg/day) based on decreased body weight gain, decreased absolute brain weight, and brain morphometric changes. The offspring NOAEL is 500 ppm (39 mg/kg/day).**

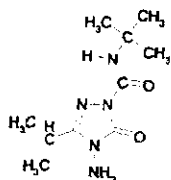
This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) pending receipt and review of the brain morphometric data for the corpus callosum for the low and mid dose groups, and review of the positive control data.

COMPLIANCE: Signed and dated Flagging, GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material:	Technical grade MKH 3586
Description:	White powder
Lot/Batch #:	05362/0005
Purity:	97.8-98.6 % a.i.
Compound Stability:	Confirmed for 15 months
CAS # of TGAI:	129909-90-6



2. **Vehicle and/or positive control:** corn oil in the diet

3. **Test animals (P):**

Species:	Rat
Strain:	Wistar Crl:W(HAN)BR
Age at study initiation:	females: at least 12 wks; males: at least 15 weeks (breeders only)
Wt. at study initiation:	177.9-287.3 g
Source:	Charles River Laboratories
Housing:	Individually or with litter in stainless steel grid or plastic cages
Diet:	Purina Mills Rodent Lab Chow 5001-4, <i>ad libitum</i>
Water:	Tap water, <i>ad libitum</i>
Environmental conditions:	Temperature: 19-25°C Humidity: 30-70% Air changes: Not stated Photoperiod: 12 hrs dark/12 hrs light
Acclimation period:	At least 6 days

B. **PROCEDURES AND STUDY DESIGN:**

1. **In life dates:** Start: March 15, 1999; End: July 20, 1999
2. **Study schedule:** The maternal animals were mated and assigned to study. The test substance was administered to the maternal animals from gestation day 0 through lactation day 21. Pups were weaned on postnatal day 21, after which time maternal animals were killed. This study was conducted in a "two-block" design with approximately half of the animals coming from each block and the second block beginning four weeks after the first block. The study included 30 parent females/dose level. F1 pups remained on study up to postnatal days 70-80 (study termination for main study).
3. **Mating procedure:** Females were paired 1:1 with males of the same strain and source. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day 0. After successful mating, each pregnant female was placed into an individual plastic nesting cage, where the dam was maintained through gestation and lactation. Females not found sperm positive within the five-day mating period were sacrificed without necropsy.
4. **Animal assignment:** Mated females and offspring were allocated as shown in Table 1 using an animal allocation program written in SAS. For offspring, four sets of animals (designated

sets A-D) were utilized for assessment at each age. Randomly-selected pups (10/sex/dose) were designated as Set D and were perfused with fixative and brains were collected for histopathological examination and morphometric analysis. Additional randomly selected pups (10/sex/dose) were selected for thyroid collection for micropathologic examination.

One pup/sex/litter/group was allocated on postnatal day 4 to one of the following: motor activity, acoustic startle habituation, passive avoidance, water maze, detailed observational battery, and sacrifice and brain examination on postnatal day 21. At approximately 6-7 weeks of age, a minimum of 10 offspring/sex/dose level from sets A, B, and C were given an Ophthalmoscopic examination. On day 70-80, these animals were sacrificed by perfusion and neural and muscle tissues collected for microscopic examination.

TABLE 1. Study design					
Experimental parameter		Dose (ppm in diet)			
		0	100	500	1000
Maternal animals--Main study					
		No. of maternal animals assigned			
FOB (GD 6, 13, 20; LD 4, 11, 21)		10	10	10	10
Offspring-- Main study					
Set A	Motor activity (PND 13, 17, 21, 58-62)	16/sex	16/sex	16/sex	16/sex
Set B	Acoustic startle habituation (PND 22, 36-40, 58-62)	16/sex	16/sex	16/sex	16/sex
Set C	Passive Avoidance (PND 22, 29)	16/sex	16/sex	16/sex	16/sex
	Detailed clinical/FOB (PND 4, 11, 21, 35, 45, 60)	16/sex	16/sex	16/sex	16/sex
	Water maze (PND 58-62, 7 days after first test. These were the same animals assigned to the passive avoidance test)	16/sex	16/sex	16/sex	16/sex
Sets A-C	Ophthalmologic evaluation (PND 50-60)	10/sex	10/sex	10/sex	10/sex
	Brain Weight (PND 70-80)	10/sex	10/sex	10/sex	10/sex
	Thyroid- Blood T3 & T4 and tissue for microscopic examination (PND 70-80)	10/sex	10/sex	10/sex	10/sex
Set D	Gross necropsy and Brain Measurements (PND 11)	10/sex	10/sex	10/sex	10/sex
	Thyroid- Blood T3 & T4 and tissue for microscopic examination (PND 11)	10/sex	10/sex	10/sex	10/sex

5. **Dose selection rationale:** Dose levels were chosen based on the results from a two-generation reproduction study in Sprague-Dawley rats (Report 108188, 1988, MRID 45121625). In that study, MKH 3586 was administered in the diet at levels of 0, 100, 500, and 1000 ppm. Effects at 1000 ppm included decreased body weight gain and increased liver weight in parental and F1-generation males and females. Body weight gain was also decreased in males and females at 500 ppm. Decreased pup weight was noted at 500 and 1000 ppm for both generations. There were no other compound-related effects. The liver was considered a target organ, with effects on the thyroid being secondary to induction of hepatic enzymes. Based on these results, the doses selected for the developmental

neurotoxicity study were 0, 100, 500, and 1000 ppm. The 100 ppm dose was selected to produce no signs of toxicity, and the 500 ppm level was selected as an intermediate dose to assist in establishing compound-related effects and a NOAEL. The 1000 ppm dose was selected to produce evidence of toxicity and approximate the MTD.

6. **Dosage administration:** MKH 3686 was administered to parent female Wistar rats in the diet at levels of 0, 100, 500 or 1000 ppm from gestation day 0 through postnatal day 21. Table 2A in the results section illustrates the mean compound intake during both the gestation and lactation phases of this study.
7. **Dosage preparation and analysis:** Detailed descriptions of feed preparations and test diet analysis were not provided; however, information from the study report is as follows. Corn oil was used as the vehicle for the test article at 1% by weight of the diet, and ethanol served as a solvent in the diet preparation process and was allowed to evaporate. Concentrations of the test substance in the diet were measured by liquid chromatography three times during the in-life phase of the study. Homogeneity and stability data from a previous study (utilizing concentrations of 20 to 10,000 ppm) were presented. References MRID 45121619 (1998), 45121620 (1998), 45121622 (1995), 45121623 (1998) and 45121624 (1994) reviewed under TXR # 0050559.

Results:

Homogeneity and stability analysis: were not performed for this study; however the study report states, "At nominal concentrations of 2 ppm and 10,000 ppm, MKH 3586 is homogeneously distributed and stable for at least 7 days at room temperature and 28 days at freezer storage conditions."

Concentration analysis: The mean analytical concentrations of test solutions were 0, 89.0, 451.7, and 939.5 ppm, respectively, for the 0, 100, 500, and 10,000 ppm diets.

C. OBSERVATIONS

1. In-life observations:

- a. **Maternal animals:** Once daily checks for mortality or moribundity and daily cage-side observations were conducted for maternal animals.

Ten dams per group were observed (by observers blind to the treatment group) in the home cage, during handling, and outside the home cage in an open field during the gestation dosing period (days 6, 13 and 20) and during the lactation dosing period (days 4, 11 and 21). The following functional observations were recorded.

Functional observations—Maternal animals

X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalmos, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Individual maternal body weight and food consumption data were recorded weekly for gestation days 0-6, 6-13, 13-20, and lactation days 0-7, 7-14, and 14-21.

From gestation day 20, dams were checked daily for evidence of parturition. They were permitted to deliver and rear offspring until postnatal day 21. Numbers of live and dead offspring were recorded during parturition.

b. Offspring:

1. **Litter observations:** Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity.

On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); excess pups were killed and discarded.

2. **Developmental landmarks:** Beginning on postnatal day 38, male offspring were examined daily for balano preputial separation. Beginning on postnatal day 29, female offspring were examined daily for vaginal patency. The age of onset was recorded.
3. **Detailed observations:** Offspring were examined for clinical signs once daily during the preweaning period and once weekly after weaning by observers aware of assignment to the treatment groups. Individual offspring body weight data were recorded on postnatal days 0, 4, 11, 17, and 21 and once weekly thereafter. Individual food consumption was measured weekly from the week of postnatal day 28.
4. **Neurobehavioral evaluations:** Observations and the schedule for those observations are summarized as follows from the report.
5. **Functional observational battery (FOB):** On postnatal days 4, 11, 21, 35, 45, and 60, a total of 16 offspring/sex/group (one male or one female from each litter) was examined outside the home cage in an FOB assessment by observers blind to the treatment groups. On postnatal days 4 and 11, the animals were not evaluated in the open field, unless deemed

necessary by the observer. Otherwise, methods were similar to the procedures used for the dams.

FUNCTIONAL OBSERVATIONS- Offspring	
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalmos, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

6. **Motor activity testing:** Motor activity was evaluated in 16/sex/dose on days 13, 17, 21, and 60. Animals were placed individually in figure-eight mazes and were continuously monitored over a 1-hour period. An automated activity monitoring system collected data over successive 10-minute intervals by recording infra-red light source break frequency within the maze. Motor activity was measured as the number of beam interruptions that occurred during the test session, and locomotor activity was measured by eliminating consecutive counts for a given beam. Therefore, only one interruption of a given beam was counted for locomotor activity until the rat relocated in the maze and interrupted a different beam. Habituation was evaluated as a decrease in activity over consecutive test-session intervals. Broad spectrum background noise ($74 \pm 2\text{dB(A)}$) was provided throughout testing to minimize acoustical variation. Uniformity of light intensity ($100 \pm 70\text{ Lux}$) over each maze was verified daily.

7. **Auditory startle habituation:** Auditory startle reflex habituation was performed on 16 offspring/sex/dose on postnatal days 22, 38 and 60, using an automated system.

Animals were acclimated for 5 minutes to background noise and were then presented with the startle stimulus at 10-second intervals for 50 trials. The startle stimulus consisted of 50-millisecond bursts of white noise at approximately 120 dB. Peak response amplitude (g force exerted on the platform) and latency (msec) measurements were recorded for each animal's individual response curve. Response amplitude was defined as the maximum value of the average curve minus the baseline (body weight). Latency to peak was defined as the time, in msec, following onset of the stimulus when the peak response amplitude occurred.

8. **Learning and memory testing:**

PASSIVE AVOIDANCE CONDITIONING: On postnatal days 24 and 31, learning and short- and long-term retention were assessed in a passive avoidance test of 16

offspring/sex/dose. Testing was done in individual isolation cubicles each with a single shuttle cage. Each cubicle was insulated to attenuate sound and had a fan for ventilation. Each 7 x 7 inch shuttle cage was separated into two equal-sized compartments by a centrally-located sliding door. The two compartments were identical except that the walls in one compartment were lined with black film (dark side) and the walls in the other compartment were not lined and this compartment was illuminated with a high-intensity lamp. The lamp was switched on at the beginning of each trial and remained on until the rat crossed into the dark compartment or the trial ended. The cage floor was constructed of a stainless steel grid and the movement of the rat from the light to dark side was detected by a photocell. Rats were placed individually into the shuttle cage facing toward the light. After 20 seconds, the light was switched on and the door separating the compartments was opened. When the rat crossed into the dark side, the door closed, a brief, mild shock (0.5 sec, 0.5mA) was delivered, and the light was switched off. If the rat failed to cross to the dark side within 180 seconds, it was returned to the holding cage and assigned a latency time of 180 sec. The procedure was repeated until the rat either remained in the bright side for 180 seconds for two consecutive trials or until 15 trials had elapsed (whichever occurred first). Rats that failed to reach criterion performance within 15 trials or failed to cross during the first two acquisition trials were excluded from the retention phase of the experiment.

WATER MAZE: Learning and memory testing was performed in 16 offspring/sex/dose on postnatal days 60 and again seven days later using an M-water maze. Only rats that demonstrated acquisition on the first test occasion were tested for retention seven days later. The water maze was made of opaque Plexiglas with 5-inch wide corridors. The walls were 16-inches high with approximately 7.5 inches of water. The maze was filled with water at $22 \pm 1^\circ\text{C}$. For each test trial, the rat was placed at the base of the M-maze stem, between the two lateral arms. On the learning trial (first trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and was then removed from the maze. The initial arm chosen on the learning trial was designated the incorrect goal during the subsequent trials (15 maximum). Rats failing to make a correct goal choice within 60-seconds in any given trial were led to the correct goal with the exit ramp and then removed from the water. The inter trial interval was approximately 15 seconds. Each rat was required to reach a criterion of 5 consecutive error-less trials to stop the test session. Latency (in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as well as the number of errors (incorrect turns) during each trial.

9. Ophthalmology At 6-7 weeks of age, indirect ophthalmoscopy was performed on 10 offspring/sex/dose (that had been selected for perfusion) following dilation with a mydriatic agent.

10. Postmortem observations:

- a. Maternal animals: Maternal animals were sacrificed by carbon dioxide inhalation on postnatal day 21. Adult females were not routinely subjected to a gross necropsy. Maternal animals found moribund were sacrificed. Those found moribund or dead were

subjected to a macroscopic necropsy, with possible collection of tissues at the discretion of the study director.

- b. **Offspring:** The offspring selected for brain weight or neuropathological evaluation were sacrificed on postnatal day 11 (Set D) or 70-80 (from sets A-C). These animals were subjected to postmortem examinations as described below.

PND 11

At postnatal day 11 one half of the Set D pups (10/sex/group) were subjected to gross necropsy and brain weight measurements. Animals were sacrificed by intra cranial injection of Fatal Plus and thyroid tissue was removed and fixed in 10% buffered formalin. After fixation, thyroid tissue was embedded in paraffin, sectioned at 5 μ m and stained with hematoxylin and eosin for microscopic examination. The livers were also removed, weighed, and collected for possible histopathological and clinical chemistry analyses (if deemed necessary to assist in interpretation of potential thyroid effects). The liver was stored in buffered 10% formalin for histology and frozen at -70 degrees for clinical chemistry.

Assessment for brain measurements from other half of the Set D pups on PND 11 was done by the following severing the head at the base of the skull and with the following procedure. The calvaria were sliced at the top of the skull to expose the brain and the entire head was immersed in 10% buffered formalin for 24 hours. The brain with olfactory bulbs was removed and weighed. Anterior to posterior cerebrum and cerebellum length were measured using a Vernier caliper.

After the gross brain measurements were recorded, brains from all dose groups were embedded in paraffin but only the control and high-dose groups were further sectioned. Tissues were sectioned at 5 μ m and stained with hematoxylin and eosin, luxol fast blue/cresyl violet (for myelin) and Sevier-Munger stains. Eight coronal sections from control and high-dose animals were examined microscopically.

The following brain morphometric measurements were performed:

- Frontal cortex thickness (dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm)
- Parietal cortex thickness (dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm)
- Caudate putamen and underlying globus pallidus diagonal width (coronal section taken at the level of the optic chiasm)
- Corpus callosum (thickness at the midline)
- Hippocampal gyrus (greatest dorsal-ventral thickness)
- Cerebellum (roof of the fourth ventricle to the dorsal surface)

On postnatal day 70-80 (Sets A-C), 10 animals/sex/group were euthanized by carbon dioxide asphyxiation, underwent a gross necropsy and their brains were removed and weighed (fresh weight); additionally, thyroid tissue was removed, weighed, fixed in 10% buffered formalin, and processed for microscopic examination. Another 10 rats/sex/dose were sacrificed by intraperitoneal injection of pentobarbitol (50 mg/kg) and perfused via the left ventricle with a sodium nitrite flush followed by fixation with 10% buffered formalin). The brain, spinal cord, both eyes with optic nerves, peripheral nerves, gasserian ganglion, gastrocnemius muscle, and both forelimbs were collected, weighed (brain only), and post-fixed with 10% buffered formalin. Anterior to posterior cerebrum and cerebellum length were measured using a Vernier caliper.

The following central and peripheral nervous tissues were dissected and preserved in paraffin (CNS tissues) or plastic (PNS tissues): eight coronal sections of the brain, cervical, thoracic, and lumbar sections of the spinal cord, the cauda equina, eyes, optic nerves, gastrocnemius muscle, dorsal root ganglia and fibers, and gasserian ganglion. Tissues from all dose groups were embedded; however, only control and high-dose tissues were examined unless effects warranted examination of low- and mid-dose samples. Paraffin-embedded tissues were sectioned at 5 μ m and stained with hematoxylin and eosin. Plastic-embedded tissues were sectioned at 2-3 μ m and stained with a modified Lee's stain. Assessments included:

Detailed morphometric evaluation of the neocortex, hippocampus, and cerebellum was conducted as follows:

- Frontal cortex thickness (dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm)
- Parietal cortex thickness (dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm)
- Caudate putamen and underlying globus pallidus diagonal width (coronal section taken at the level of the optic chiasm)
- Corpus callosum (thickness at the midline)
- Hippocampal gyrus (greatest dorsal-ventral thickness)
- Cerebellum (roof of the fourth ventricle to the dorsal surface)

T3 and T4 measurements: On PND 11, blood T3 and T4 levels were measured from blood samples collected via decapitation from 10 pups/sex/dose from Set D. On PND 75, blood T3 and T4 levels were measured from blood samples collected via the orbital plexus from randomly selected pups (10 pups/sex/dose) from Sets A-C.

D. DATA ANALYSIS:

1. **Statistical analyses:** Continuous data were initially analyzed for equality of variance using Bartlett's test. Group means with equal variances were further analyzed with ANOVA, followed by Dunnett's test if significance was identified with the ANOVA. Group means with unequal variances were analyzed by Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons. The level of significance was set at $p \leq 0.05$, except for Bartlett's test which was set at $p \leq 0.01$.

Motor and locomotor activity were analyzed with ANOVA, followed by Dunnett's test if significance was attained with ANOVA. Acoustic startle peak amplitude data were analyzed by ANOVA followed by Dunnett's test if significance was observed with the ANOVA. The response amplitude data for each block of 10 trials were subjected to a Repeated-Measures ANOVA, using the test block as the repeated measure. Passive avoidance latency data and Water maze data were analyzed by a univariate ANOVA followed by Dunnett's test. Micropathology frequency data were analyzed by Chi-Square followed by Fisher's Exact Test if significance was identified with the Chi-Square.

2. **Indices:**

- a. **Reproductive indices:** The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Gestation index = (Number of females with live pups/Number pregnant) \times 100

Mating index = (Number of inseminated females/Number of females co-housed with males) \times 100

Fertility index = (Number of pregnant females/Number of inseminated females) \times 100

- b. **Offspring viability indices:** The following viability (survival) indices were calculated from lactation records of litters in the study:

Live birth index = (Number of live pups born per litter/Total number of pups per litter) \times 100

Viability index = (Number of live offspring at PND 4/Number of live offspring at PND 1) \times 100

Lactation index = (Number of live offspring on Day 21/Number of live offspring on PND 4 after culling) \times 100

3. **Positive and historical control data:** HED has on file a battery of four positive control studies from the Bayer Stilwell, Kansas Laboratory as follows:

2001, MRID 45441302. Methimazole

2001, MRID 45441303. Test for validation of auditory startle habituation and cognitive function (passive avoidance and water maze).

1999, MRID 45464601. Personal training in the FOB

2000, MRID 45464602. Motor activity and method validation with triadimfon and chlorpyrifos.

II. RESULTS:**A. PARENTAL ANIMALS:**

1. **Mortality and clinical and functional observations:** There were no maternal deaths before scheduled termination, and there were no treatment-related clinical signs observed during gestation or lactation. There was an increase ($p < 0.05$) in the number of fecal boli for high-dose dams on GD6 compared to controls. This effect is of questionable toxicological significance and no other treatment-related effects were noted during the abbreviated FOB.
2. **Body weight, food consumption and compound intake:** Selected group mean body weights and food consumption values and calculations for feed efficiency for pregnant or nursing dams are summarized in Table 2. During gestation, maternal body weight of animals fed 1000 ppm was decreased ($p < 0.05$) an average of 5% on GD 6, with differences persisting to GD 20. An average 12% decrease ($p < 0.05$) in body weight gain was noted from GD 0 to GD 20 for high-dose dams compared to controls. During lactation, maternal body weight was decreased for high-dose rats compared to controls; decreases averaged 4% on LD 0 to 9% on LD 14 and LD 21 ($p < 0.05$ for LD 4, 11, 14, and 21). Body weight gain during lactation was decreased 27% at 500 ppm and 44% at 1000 ppm compared to controls.

There were apparent treatment-related effects on food consumption during gestation and lactation. The increase in food consumption for mid and high-dose dams for PND 14-21 together with the relatively lower body weight for these animals indicated an effect on food efficiency in the dams for these groups.

TABLE 2. Selected Mean (\pm SD) maternal body weight and food consumption ^a				
Observations/study interval	Dose (ppm)			
	0	100	500	1000
Gestation (n= 27-29)				
Body wt. Gestation day 0 (g)	227.8 \pm 2.90	221.3 \pm 3.66	232.3 \pm 4.08	226.3 \pm 3.45
Body wt. Gestation day 6 (g)	247.2 \pm 3.18	243.4 \pm 3.18	248.8 \pm 4.19	234.3* \pm 3.80(15%)
Body wt. Gestation day 13 (g)	271.3 \pm 3.30	265.9 \pm 3.57	272.7 \pm 4.37	258.7 \pm 4.23(15%)
Body wt. Gestation day 20 (g)	333.2 \pm 4.56	325.0 \pm 5.03	333.8 \pm 5.89	319.6 \pm 5.49(14%)
Wt. gain gestation days 6-20 (g)	105.4 \pm 2.75	103.7 \pm 2.79	101.5 \pm 3.00	92.8* \pm 3.21(112%)
Food consumption gestation days 0-6 (g/kg/day)	87.0 \pm 2.60	86.7 \pm 1.84	85.7 \pm 2.14	90.7 \pm 5.19
Food consumption gestation days 6-13 (g/kg/day)	93.4 \pm 2.90	90.6 \pm 1.98	92.3 \pm 2.88	109.2 \pm 9.47
Food consumption gestation days 13-20 (g/kg/day)	82.4 \pm 1.69	82.6 \pm 1.13	82.8 \pm 1.09	90.2* \pm 2.19(19.5%)
Food consumption gestation days 6-20 (g/kg/day)#	175.8	173.2	175.1	199.4(113%)
Food efficiency (days 6-20)	0.60	0.60	0.58	0.47(122%)
Lactation (n=23-29)				

TABLE 2. Selected Mean (\pm SD) maternal body weight and food consumption ^a				
Observations/study interval	Dose (ppm)			
	0	100	500	1000
Body wt. lactation day 0 (g)	255.7 \pm 3.60	250.3 \pm 3.98	254.8 \pm 4.00	244.4 \pm 3.21
Body wt. lactation day 4 (g)	269.4 \pm 3.62	263.5 \pm 4.97	266.4 \pm 4.82	251.7** \pm 4.45(7%)
Body wt. lactation day 7 (g)	271.7 \pm 4.51	269.2 \pm 4.74	270.4 \pm 4.46	258.6 \pm 4.30(5%)
Body wt. lactation day 11 (g)	287.7 \pm 3.93	282.5 \pm 5.17	285.7 \pm 4.48	268.0** \pm 4.30(7%)
Body wt. lactation day 14 (g)	288.2 \pm 3.51	280.2 \pm 5.65	281.6 \pm 4.40	263.6** \pm 4.05(9%)
Body wt. lactation day 21(g)	287.1 \pm 3.25	281.9 \pm 4.34	277.7 \pm 5.24	262.0** \pm 5.05(9%)
Body wt. gain (0-21) (g)	31.8	31.6	22.9(128%)	17.6(145%)
Food consumption lactation days 0-7 (g/kg/day)	144.4 \pm 8.74	160.4 \pm 9.20	142.6 \pm 5.16	149.1 \pm 8.28
Food consumption lactation days 7-14 (g/kg/day)	180.5 \pm 3.15	183.1 \pm 3.23	202.3* \pm 7.74	191.3 \pm 5.58
Food consumption lactation days 14-21 (g/kg/day)	198.6 \pm 3.27	211.4 \pm 4.95	213.6 \pm 6.94	225.7** \pm 6.6(14%)
Food consumption lactation days 0-21 (g/kg/day)#	523.9	554.9	558.5(17%)	566.1(18%)
Food efficiency (days 0-21)##	0.061	0.057	0.041(133%)	0.031(149%)

^aData obtained from Tables 3 & 4 pages 62-65 and Tables 6 & 7 pages 68-71, MRID 45441301. *p<0.05, **p<0.01

#Calculated from preceding rows in table by EPA reviewer. For gestation sum of interval 6-13 and 13 to 20. For lactation, sum of 0-7, 7-14 and 14-21. Ratio of the feed consumed to body weight gain from days 6-20 for gestation and for days 0-21 for lactation.

Intake of the test material varied during both gestation and lactation as shown in Table 2A and during lactation, there was about a 50% greater amount of compound consumed in the last week of lactation as compared with the first week of lactation. Table 2A also shows the approximate mean calculated by the EPA reviewer for both gestation and lactation.

Table 2A. Compound intake in mg/kg/day in dams during gestation and lactation.

Interval (Day)	Dose Group (ppm)			
	Control	100	500	1000
Gestation				
0-6	0	7.8 \pm 0.17	38.7 \pm 0.97	85.2 \pm 4.88
6-13	0	8.1 \pm 0.18	41.7 \pm 1.3	102.6 \pm 8.9
13-20	0	7.4 \pm 0.10	37.4 \pm 0.49	84.7 \pm 2.05
mean*	0	~8	~39	~91
Lactation				
0-7	0	14.4 \pm 0.83	64.4 \pm 2.33	140.1 \pm 7.78
7-14	0	16.4 \pm 0.29	91.4 \pm 3.49	179.7 \pm 5.24
14-21	0	19.0 \pm 0.44	96.5 \pm 3.13	212.0 \pm 6.21
mean*	0	~17	~84	~177

*Expressed as rounded number. ~ used to indicate the approximate nature of the data.

Data are from pages 73 and 74 of the study report.

3. **Reproductive performance:** Results for the maternal animals are summarized in Table 3. No treatment-related effects were noted.

TABLE 3. Reproductive performance ^a				
Observation	Dose (ppm)			
	0	100	500	1000
Number Mated	30	30	30	30
Number Delivered	29	28	28	29
Mating Index (%)	100	100	100	100
Fertility Index (%)	96.7	93.3	93.3	96.7
Gestation index (%)	100	100	100	100

^aData obtained from Table 1, pages 58-59, MRID 45441301.

B. OFFSPRING

1. **Viability and clinical signs:** Litter size and viability (survival) results from pups during lactation are summarized in Table 4. There was no treatment-related effect on the number of litters, live litter size, number of stillborn pups, live birth index, or viability index.

It is noted that the number of dams with pups delivered in Table 3 above differs slightly from the number of litters in Table 4. This is because some of the dams from each litter were sacrificed without a clear explanation although it was noted that some of the dams had less than the desirable number of pups.

TABLE 4. Litter size and viability ^a				
Observation	Dose (ppm)			
	0	100	500	1000
Number of Litters#	28	25	26	24
Total number born	326	284	304	267
Number born live	326	283	304	267
Number born dead	0	1	0	0
Mean No. of viable pups:				
Day 0	12	11	12	11
Day 4 ^b	12	11	12	11
Day 4 ^c	8	8	8	8
Day 21	6	6	6	6
Mean Live birth index (%)	99.6	98.9	99.6	100.0
Mean Viability index	99.7	99.6	99.2	99.7
Mean Lactation index	75.4	75.0	75.8	75.0

^aData obtained from Table 9, pages 75-77, MRID 45441301.

^bBefore standardization (culling).

^cAfter standardization (culling).

#Some litters were not included and were "Elected sacrifice" but the cause or purpose was not further described.

2. **Body weight:** Body weights were comparable at birth and on PND 4 across all dose groups; however, by PND 11, body weight was decreased 7-8% ($p<0.05$) for high-dose male and female offspring; this decrease averaged 11-12% ($p<0.01$) by weaning on PND 21. Body weight gain was decreased in males and females in the 500 (8-13%; $p<0.05$ or $p<0.01$) and 1000 ppm (11-21%; $p<0.05$ or $p<0.01$) groups. No treatment-related effects on body weight gain were noted in low-dose pups. Selected mean preweaning pup body weight data are presented in Table 5.

TABLE 5. Mean (\pm SD) pre-weaning pup body weights and body weight Gain (g) ^a								
Postnatal Day	Dose (ppm)							
	0	100	500	1000	0	100	500	1000
	Males				Females			
0	5.9 \pm 0.09	5.7 \pm 0.08	6.1 \pm 0.09	5.8 \pm 0.07	5.6 \pm 0.09	5.4 \pm 0.09	5.7 \pm 0.07	5.6 \pm 0.07
4 ^b	9.4 \pm 0.18	9.3 \pm 0.16	9.5 \pm 0.19	9.0 \pm 0.17	9.0 \pm 0.19	9.0 \pm 0.16	9.1 \pm 0.15	8.7 \pm 0.17
4 ^c	9.4 \pm 0.18	9.3 \pm 0.17	9.6 \pm 0.20	9.0 \pm 0.17	9.1 \pm 0.20	9.1 \pm 0.15	9.2 \pm 0.15	8.7 \pm 0.18
11	21.7 \pm 0.40	21.9 \pm 0.36	21.7 \pm 0.42	20.1* \pm 0.42 (7%) ^d	21.2 \pm 0.42	21.2 \pm 0.37	21.1 \pm 0.41	19.6* \pm 0.44 (8%) ^d
17	36.1 \pm 0.61	36.0 \pm 0.56	34.9 \pm 0.55	32.6* \pm 0.63 (10%) ^d	35.1 \pm 0.58	34.8 \pm 0.57	33.9 \pm 0.54	32.0** \pm 0.62 (9%) ^d
21	46.3 \pm 0.64	46.1 \pm 0.69	43.9 \pm 0.78	40.7** \pm 0.96 (12%) ^d	44.6 \pm 0.62	44.2 \pm 0.65	42.6 \pm 0.72	39.9** \pm 0.78 (11%) ^d
Weight gain Days 0-4	3.5 \pm 0.12	3.6 \pm 0.11	3.4 \pm 0.14	3.2 \pm 0.12	3.4 \pm 0.13	3.6 \pm 0.09	3.4 \pm 0.12	3.1 \pm 0.12
Weight gain Days 4-11	12.4 \pm 0.33	12.5 \pm 0.29	12.1 \pm 0.33	11.1* \pm 0.35 (11%) ^d	12.2 \pm 0.33	12.1 \pm 0.29	11.9 \pm 0.34	10.9* \pm 0.34 (11%) ^d
Weight gain Days 11-17	14.3 \pm 0.30	14.1 \pm 0.29	13.2* \pm 0.25 (8%) ^d	12.6** \pm 0.34 (12%) ^d	13.9 \pm 0.22	13.7 \pm 0.29	12.8** \pm 0.27 (8%) ^d	12.5** \pm 0.27 (10%) ^d
Weight gain Days 17-21	10.3 \pm 0.21	10.1 \pm 0.31	9.0** \pm 0.34 (13%) ^d	8.1** \pm 0.43 (21%) ^d	9.5 \pm 0.20	9.4 \pm 0.25	8.7 \pm 0.35 (8%) ^d	7.8** \pm 0.31 (18%) ^d

^a Data obtained from Tables 12-13, pages 82-90, MRID 45441301. * $p<0.05$, ** $p<0.01$.

^b Before standardization (culling).

^c After standardization (culling).

^d (%) decrease compared to controls, calculated by reviewer

Body weights were decreased in mid- and high-dose males and females following weaning. For males and females in the 500 ppm group body weights were approximately 4% less than controls at day 28 (the first time following weaning). Males recovered by the following week and females by study termination. For 1000 ppm males and females, body weights were 3-10% less than controls following weaning. These differences persisted through study termination. No biologically-significant postweaning body weight effects were noted at 100 ppm. Selected mean postweaning offspring body weight data are presented in Table 6.

TABLE 6. Mean (\pm SD) post-weaning pup body weights (g)^a

Postnatal Day	Dose (ppm)							
	0	100	500	1000	0	100	500	1000
	Males				Females			
29	73.07 \pm 8.63	72.03 \pm 7.99	70.06* \pm 6.62 (4%)	65.74* \pm 8.30 (10%)	72.95 \pm 6.97	71.77 \pm 7.50	70.13* \pm 5.68 (4%)	67.83* \pm 7.49 (7%)
36	118.67 \pm 11.05	116.17 \pm 13.04	114.99 \pm 8.68	108.69* \pm 11.49 (8%)	110.84 \pm 9.07	107.88 \pm 9.70	106.67* \pm 7.47 (4%)	103.24* \pm 9.83 (7%)
43	163.17 \pm 14.75	160.99 \pm 15.90	159.87 \pm 11.90	153.80* \pm 13.5 (6%)	136.97 \pm 10.04	113.13* \pm 9.90 (17%)	132.53* \pm 8.19 (3%)	129.63* \pm 10.28 (5%)
50	205.37 \pm 18.52	202.25 \pm 18.53	201.62 \pm 14.97	194.83* \pm 16.26 (5%)	156.49 \pm 11.25	152.32 \pm 12.25	151.27* \pm 9.88 (3%)	148.33* \pm 11.83 (5%)
57	251.37 \pm 20.51	247.03 \pm 22.32	247.33 \pm 17.28	237.69* \pm 19.97 (5%)	175.15 \pm 13.42	171.12 \pm 14.11	170.16* \pm 11.20 (3%)	168.56* \pm 13.16 (4%)
64	284.40 \pm 24.17	279.24 \pm 25.23	279.69 \pm 20.23	269.61* \pm 21.00 (5%)	188.67 \pm 15.62	183.99 \pm 14.91	183.11* \pm 12.36 (3%)	180.60* \pm 13.70 (4%)
71	313.51 \pm 26.15	307.60 \pm 27.23	307.73 \pm 23.29	298.00* \pm 24.75 (5%)	198.73 \pm 17.22	194.31 \pm 16.56	193.69 \pm 13.68	191.77* \pm 14.85 (4%)

^a Data obtained from Table 15, pages 93-95, MRID 45441301. *p<0.05

Number in parentheses = % decrease compared to controls, calculated by reviewer

The body weight gain effects during lactation were considered transitory in the 500 ppm dose group and not an true adverse effect.

No treatment-related postweaning food consumption effects were noted.

3. Developmental landmarks:

- a. **Sexual maturation:** Preputial separation for high-dose males was not delayed relative to controls. There were no treatment-related effects on the mean age for attainment of vaginal opening for females. The data are presented in Table 7.

TABLE 7. Mean (\pm SD) age of sexual maturation (days)^a

Parameter	Dose (ppm)			
	0	100	500	1000
N (M/F)	28/28	25/25	26/26	24/24
Preputial separation (males)	45.4 \pm 0.36	44.1 \pm 0.40	45.0 \pm 0.37	45.6 \pm 0.39
Vaginal opening (females)	33.8 \pm 0.35	33.8 \pm 0.30	33.4 \pm 0.32	34.0 \pm 0.38

^a Data obtained from Table 14, pages 91-92, MRID 454441301.

- b. **Surface Righting, eye opening, auditory startle, and pupil constriction:** No treatment-related effects were noted with regard to these developmental landmarks.

4. Behavioral assessments:

- a. **Functional observational battery:** There were no treatment-related effects at any dose level on any test day (PND 4, 11, 21, 35, 45, or 60).
- b. **Motor activity:** No treatment-related overall or interval motor or locomotor activity effects were noted. For motor activity in control males and females, habituation was apparent on all four test days. For locomotor activity, habituation was achieved for treated rats on all test days, even on PND 13 when activity was low due to the developmental stage of the rats. Habituation was not distinctly apparent for control rats on PND 13, due to extremely low activity. Total activity data are presented in Tables 8 and 9.

TABLE 8. Mean (\pm S.D.) motor activity data (total activity counts for session) ^a				
Test Day	Dose (ppm)			
	0	100	500	1000
Males				
PND 13	158 \pm 127	158 \pm 124	204 \pm 167	162 \pm 137
PND 17	365 \pm 286	353 \pm 211	421 \pm 222	341 \pm 212
PND 21	495 \pm 228	459 \pm 201	520 \pm 229	491 \pm 184
PND 60	650 \pm 188	603 \pm 170	634 \pm 188	682 \pm 182
Females				
PND 13	160 \pm 107	152 \pm 92	179 \pm 148	144 \pm 105
PND 17	370 \pm 207	357 \pm 194	403 \pm 325	313 \pm 241
PND 21	463 \pm 155	495 \pm 185	506 \pm 194	459 \pm 246
PND 60	889 \pm 192	867 \pm 267	859 \pm 254	890 \pm 246

^a Data obtained from Table 19, pages 197-199, MRID 454441301

N = 24-28/sex/dose.

TABLE 9. Mean (\pm S.D.) locomotor activity data (total activity counts for session) ^a				
Test Day	Dose (ppm)			
	0	100	500	1000
Males				
PND 13	10 \pm 23	18 \pm 38	24 \pm 39	16 \pm 25
PND 17	68 \pm 52	66 \pm 41	85 \pm 52	63 \pm 40
PND 21	101 \pm 41	82 \pm 28	102 \pm 32	108 \pm 30
PND 60	363 \pm 105	350 \pm 107	358 \pm 102	393 \pm 91
Females				
PND 13	14 \pm 20	16 \pm 18	21 \pm 35	16 \pm 20
PND 17	67 \pm 46	66 \pm 39	80 \pm 72	64 \pm 46
PND 21	102 \pm 32	100 \pm 26	114 \pm 39	105 \pm 42
PND 60	441 \pm 97	429 \pm 150	426 \pm 101	439 \pm 125

^a Data obtained from Table 20, pages 200-202, MRID 454441301.

N = 24-28/sex/dose.

- c. **Auditory startle reflex** : There were no effects on startle response amplitude at any dose and there was no treatment-related effect noted for peak latency at any dose on any test day. Habituation was evident in control males and females on all test days as a decrease in response amplitude over the test session. Peak amplitude data are summarized in Table 10 and latency data are summarized in Table 11.

TABLE 10. Auditory startle reflex peak amplitude data (mean g \pm S.D.) ^a						
		Trial Block	Dose (ppm)			
			0	100	500	1000
Males						
PND 22	1	43 \pm 14	45 \pm 14	36 \pm 13	36 \pm 11	
	2	44 \pm 18	46 \pm 18	41 \pm 16	39 \pm 16	
	3	42 \pm 20	43 \pm 18	41 \pm 14	35 \pm 12	
	4	40 \pm 19	41 \pm 15	37 \pm 13	32 \pm 14	
	5	36 \pm 19	34 \pm 14	33 \pm 12	30 \pm 13	
	Mean	41 \pm 17	42 \pm 14	37 \pm 12	34 \pm 11	
PND 38	1	132 \pm 75	123 \pm 53	133 \pm 76	145 \pm 71	
	2	124 \pm 69	117 \pm 60	130 \pm 84	144 \pm 85	
	3	105 \pm 55	109 \pm 56	111 \pm 64	127 \pm 75	
	4	90 \pm 46	91 \pm 59	94 \pm 55	99 \pm 63	
	5	79 \pm 43	78 \pm 47	88 \pm 49	94 \pm 48	
	Mean	106 \pm 51	104 \pm 50	111 \pm 60	122 \pm 64	
PND 60	1	346 \pm 163	315 \pm 132	439 \pm 194	396 \pm 211	
	2	299 \pm 176	290 \pm 158	400 \pm 209	363 \pm 214	
	3	273 \pm 165	234 \pm 128	350 \pm 214	325 \pm 214	
	4	231 \pm 159	189 \pm 68	280 \pm 188	267 \pm 180	
	5	215 \pm 161	185 \pm 110	270 \pm 154	237 \pm 182	
	Mean	273 \pm 153	243 \pm 105	348 \pm 178	318 \pm 193	
Females						
PND 22	1	39 \pm 14	43 \pm 16	45 \pm 16	38 \pm 15	
	2	39 \pm 16	40 \pm 18	45 \pm 14	36 \pm 15	
	3	35 \pm 13	36 \pm 17	42 \pm 19	32 \pm 17	
	4	31 \pm 12	33 \pm 15	39 \pm 19	29 \pm 16	
	5	28 \pm 10	32 \pm 15	37 \pm 19	25 \pm 13	
	Mean	35 \pm 11	37 \pm 15	42 \pm 16	32 \pm 14	
PND 38	1	96 \pm 55	108 \pm 65	105 \pm 68	91 \pm 66	
	2	107 \pm 79	109 \pm 70	107 \pm 59	87 \pm 52	
	3	86 \pm 51	88 \pm 66	91 \pm 49	80 \pm 54	
	4	72 \pm 35	75 \pm 45	74 \pm 42	69 \pm 48	
	5	63 \pm 31	70 \pm 40	63 \pm 35	64 \pm 40	
	Mean	85 \pm 42	90 \pm 52	88 \pm 47	78 \pm 48	
PND 60	1	155 \pm 86	216 \pm 153	213 \pm 121	178 \pm 121	
	2	178 \pm 112	222 \pm 171	239 \pm 143	189 \pm 139	
	3	129 \pm 83	175 \pm 124	192 \pm 110	161 \pm 109	
	4	88 \pm 52	129 \pm 89	149 \pm 120	113 \pm 92	

TABLE 10. Auditory startle reflex peak amplitude data (mean g \pm S.D.)^a

	Trial Block	Dose (ppm)			
		0	100	500	1000
	5	94 \pm 50	112 \pm 70	146 \pm 89	108 \pm 72
	Mean	129 \pm 67	171 \pm 113	188 \pm 107	150 \pm 99

^aData obtained from Tables 23-24, pages 221-230, MRID 45441301

N = 23-28/sex/dose

TABLE 11. Auditory startle latency to peak data (mean msec \pm S.D.)^a

	Trial Block	Dose (ppm)			
		0	100	500	1000
Males					
PND 22	1	39±9	39±10	42±9	41±7
	2	35±8	34±7	41±11	37±8
	3	35±7	34±7	38±10	38±10
	4	36±8	34±7	40±10	41±10
	5	35±7	35±5	38±10	41±10
	Mean	36±6	35±6	40±7	40±7
PND 38	1	33±3	34±3	33±3	32±4
	2	31±3	30±3	32±4	31±3
	3	30±3	31±4	32±4	32±4
	4	32±4	32±4	33±5	32±4
	5	34±6	33±4	33±4	32±4
	Mean	32±3	32±2	33±3	32±2
PND 60	1	36±3	37±3	36±3	37±3
	2	34±3	34±3	35±3	35±3
	3	34±3	34±2	35±2	35±4
	4	34±4	33±3	35±3	35±3
	5	34±4	35±4	35±3	34±3
	Mean	34±3	35±2	35±2	35±2
Females					
PND 22	1	42±8	42±8	44±7	45±9
	2	41±9	40±8	39±8	43±11
	3	39±7	37±7	41±10	41±9
	4	40±7	41±9	40±9	42±9
	5	40±6	41±9	41±9	41±8
	Mean	40±5	40±6	41±6	42±7
PND 38	1	34±3	36±6	35±5	35±5
	2	32±5	33±5	32±3	33±6
	3	33±5	33±5	33±5	33±6
	4	33±5	34±7	34±6	33±6
	5	34±7	36±8	35±6	35±6
	Mean	33±4	34±5	34±3	34±5
PND 60	1	38±4	40±4	39±6	40±5

TABLE 11. Auditory startle latency to peak data (mean msec \pm S.D.) ^a					
	Trial Block	Dose (ppm)			
		0	100	500	1000
	2	36 \pm 5	37 \pm 4	36 \pm 5	37 \pm 5
	3	35 \pm 5	37 \pm 5	36 \pm 4	37 \pm 6
	4	36 \pm 5	39 \pm 5	37 \pm 6	39 \pm 6
	5	37 \pm 7	38 \pm 6	37 \pm 5	38 \pm 5
	Mean	36 \pm 3	38 \pm 4	37 \pm 3	38 \pm 4

^aData obtained from Tables 23-24, pages 221-230, MRID 45441301

N = 23-28/sex/dose

d. Learning and memory testing:

Passive Avoidance: There were no treatment-related effects, and acquisition and retention were appropriate in control animals. Data are summarized in Table 12.

TABLE 12. Passive avoidance performance at PND 24/31(mean \pm S.D.) ^a					
Test Day/Parameter		Dose (ppm)			
		0	100	500	1000
Males					
Session 1 (Learning)	Trials to criterion	3.3 \pm 0.7	3.5 \pm 1.0	3.9 \pm 1.4	3.3 \pm 0.7
	Latency trial 1 (sec)	45.1 \pm 44.8	37.5 \pm 37.7	43.8 \pm 40.4	47.1 \pm 43.6
	Latency trial 2 (sec)	172.2 \pm 30.2	166.9 \pm 37.4	152.6 \pm 53.7	171.2 \pm 27.9
	Failed to Learn/No. Tested	0/28	0/25	0/26	0/24
Session 2 (Retention)	Trials to criterion	2.5 \pm 0.8	2.2 \pm 0.6	2.4 \pm 0.8	2.1 \pm 0.5
	Latency trial 1 (sec)	162.5 \pm 41.6	180.0* \pm 0.0	179.2 \pm 4.1	177.4 \pm 12.2
	Latency trial 2 (sec)	171.1 \pm 23.3	179.0 \pm 4.5	169.4 \pm 29.7	173.7 \pm 29.6
Females					
Session 1 (Learning)	Trials to criterion	3.7 \pm 1.0	3.2 \pm 0.7	3.3 \pm 0.7	3.7 \pm 1.2
	Latency trial 1 (sec)	39.6 \pm 44.0	43.4 \pm 45.5	35.2 \pm 43.5	56.8 \pm 50.9
	Latency trial 2 (sec)	154.0 \pm 47.3	173.5 \pm 32.3	162.6 \pm 41.6	167.5 \pm 35.5
	Failed to Learn/No. Tested	0/28	0/25	0/25	0/24
Session 2 (Retention)	Trials to criterion	2.2 \pm 0.4	2.4 \pm 0.7	2.4 \pm 0.7	2.4 \pm 0.7
	Latency trial 1 (sec)	162.7 \pm 42.0	166.5 \pm 37.3	158.9 \pm 44.3	168.6 \pm 25.0
	Latency trial 2 (sec)	180.0 \pm 0.0	167.8 \pm 36.9	180.0 \pm 0.0	172.9 \pm 23.0

^aData obtained from Table 25, pages 231-233, MRID 45441301. *p<0.05.

Water Maze: There were no treatment-related differences for males or females at any dose level compared to controls with regard to trials-to criterion, time to escape, number of errors, or failure to meet criterion. Data are summarized in Table 13.

TABLE 13. Water maze performance at PND 60/67					
Test Day/Parameter		Dose (ppm)			
		0	100	500	1000
Males					
Session 1 (Learning)	Trials to criterion	7.9±2.5	8.0±2.4	8.0±2.6	7.3±2.3
	Trial 1 errors (mean ± SD)	1.0±0.8	0.8±1.2	1.0±1.2	0.9±1.0
	Trial 1 duration (sec) (mean ± SD)	16.9±9.0	19.4±12.9	22.3±14.9	19.0±12.8
	Trial 2 errors (mean ± SD)	0.6±1.0	0.8±1.0	0.4±0.6	0.4±0.8
	Trial 2 duration (sec) (mean ± SD)	13.0±10.6	16.5±9.7	14.3±9.7	14.4±10.3
	Failed to meet criterion	0/28 (0%)	0/25 (0%)	0/26 (0%)	0/24 (0%)
Session 2 (retention)	Trials to criterion	5.7±1.6	5.9±1.4	6.4±2.7	6.5±2.1
	Trial 1 errors (mean ± SD)	0.4±0.8	0.8±1.4	0.1±0.6	0.8±1.3
	Trial 1 duration (sec) (mean ± SD)	7.6±5.6	11.6±10.6	7.0±9.4	10.0±7.7
	Trial 2 errors (mean ± SD)	0.1±0.4	0.2±0.6	0.1±0.4	0.2±0.6
	Trial 2 duration (sec) (mean ± SD)	5.0±2.3	5.6±2.7	4.6±2.8	5.5±3.2
Females					
Session 1 (Learning)	Trials to criterion	8.3±3.6	8.3±2.4	8.4±3.0	8.6±2.9
	Trial 1 errors (mean ± SD)	0.7±0.6	1.0±1.2	1.0±1.0	1.0±1.0
	Trial 1 duration (sec) (mean ± SD)	17.2±11.4	19.9±11.9	17.7±8.1	18.2±9.1
	Trial 2 errors (mean ± SD)	0.7±0.9	0.9±1.2	0.7±0.9	0.9±0.9
	Trial 2 duration (sec) (mean ± SD)	13.8±9.5	14.9±11.7	16.2±9.5	16.6±9.0
	Failed to meet criterion	4/28 (14%)	0/24 (0%)	2/25 (8%)	2/24 (8%)
Session 2 (retention)	Trials to criterion	7.0±3.0	7.8±3.3	7.1±2.5	8.0±3.2
	Trial 1 errors (mean ± SD)	0.5±1.7	1.0±1.8	1.0±1.8	0.7±1.3
	Trial 1 duration (sec) (mean ± SD)	7.3±9.0	11.6±10.1	12.4±10.3	10.2±9.2
	Trial 2 errors (mean ± SD)	0.2±0.6	0.2±0.6	0.6±1.3	0.3±1.3
	Trial 2 duration (sec) (mean ± SD)	5.7±4.5	6.6±4.2	7.3±6.2	6.5±5.0

a Data obtained from Table 26, pages 234-236, MRID 45441301.

- e. **Ophthalmology:** The study author asserts that there were no treatment-related ocular effects in the treated animals. Data on pages 1086 (males) and 1087 (females) indicate that 2-5 males 1-3 females have corneal opacity but there was no dose response evident.

5. **Postmortem results:**

- a. **Brain weights:** . Mean brain weight data are presented in Table 14. On PND 11, there were decreases in brain weight in males (12%) and females (7%) in the 1000 ppm group. Absolute and relative brain weights of male and female offspring were unaffected by treatment at study termination.

TABLE 14. Mean (\pm SD) Brain Weight Data in Offspring^a

Parameter	Dose (ppm)			
	0	100	500	1000
Males				
Day 11				
Terminal body weight (g)	21.2 \pm 1.5	20.5 \pm 2.0	22.3 \pm 2.4	19.2 \pm 2.9 (.9%)
Brain weight (g)	1.321 \pm 0.179	1.220 \pm 0.076 (18%)	1.255 \pm 0.056 (15%)	1.157 \pm 0.114 (112%)
Brain-to-body weight ratio	6.227 \pm 0.572	5.970 \pm 0.413	5.669 \pm 0.380	6.062 \pm 0.500
Termination				
Terminal body weight (g)	328.8 \pm 36.3	335.5 \pm 31.3	316.3 \pm 31.6	300.8 \pm 35.3 (19%)
Brain weight (g)	1.905 \pm 0.066	1.930 \pm 0.098	1.874 \pm 0.077	1.867 \pm 0.088
Brain-to-body weight ratio	0.584 \pm 0.046	0.578 \pm 0.035	0.596 \pm 0.043	0.627 \pm 0.064 (17%)
Females				
Day 11				
Terminal body weight (g)	21.6 \pm 4.2	19.8 \pm 4.7	19.6 \pm 3.6	19.3 \pm 4.3
Brain weight (g)	1.234 \pm 0.099	1.212 \pm 0.080 (12%) ^b	1.176 \pm 0.129 (15%)	1.145 \pm 0.157 (17%)
Brain-to-body weight ratio	5.874 \pm 0.961	6.420 \pm 1.547	6.090 \pm 0.546	6.046 \pm 0.674
Termination				
Terminal body weight (g)	210.5 \pm 15.0	199.2 \pm 15.9	205.7 \pm 12.8	196.1 \pm 21.0
Brain weight (g)	1.758 \pm 0.066	1.754 \pm 0.069	1.718 \pm 0.072	1.721 \pm 0.081
Brain-to-body weight ratio	0.838 \pm 0.052	0.884 \pm 0.058	0.838 \pm 0.057	0.885 \pm 0.083

^a Data obtained from pages 1161-1162 & 1167-1168, MRID 45441301.

^b Number in parentheses = % decrease compared to controls, calculated by reviewer

N = 5/sex/dose

- b. Liver and thyroid weight:** Absolute and relative liver and thyroid weights of male and female offspring were unaffected by treatment on PND 11 or at study termination. No treatment-related thyroid histopathology effects were noted. In particular, for 11 day old pups, the control group males were 0.724 \pm 0.121 and the 1000 ppm dose group was 0.737 \pm 0.098 and for females these values were 0.747 \pm 0.092 and 0.707 \pm 0.291 gms (no data were presented for day 11 thyroids).

At termination (page 1242), liver weights for males were 16.807 \pm 2.197 for the control and 14.196 \pm 1.916 for the high dose group indicating a 16% decrease. There was a corresponding decrease in liver weight to body weight of 4%. In females, the control group liver weight was 10.529 \pm 1.140 and the high dose group was 9.423 \pm 0.733 to indicate an 11% decrease. Liver to body weight ratio was similar between the control and high dose. The lower liver weight probably corresponded to the lower body weight in this group.

At termination, male thyroid weight (in gms) for the control (0.024 \pm 0.003 for males and 0.017 \pm 0.003 for females) to the high dose group (0.020 \pm 0.006 for males and 0.017 \pm 0.003 for females) and the relative to body weight ratios were also similar (0.0075 \pm 0.0009 for males and 0.008 \pm 0.0018 for females) was similar to the high dose group (0.0066 \pm 0.0019 for males and 0.0091 \pm 0.0012 for females). Thus there were no effects on thyroid weight at termination.

Liver weight data are summarized in Table 15.

TABLE 15. Mean (\pm SD) Liver Weight Data in Offspring ^a				
Parameter	Dose (ppm)			
	0	100	500	1000
Males				
Day 11				
Liver weight (g)	0.724 \pm 0.121	0.691 \pm 0.077	0.723 \pm 0.100	0.737 \pm 0.098
Liver-to-body weight ratio	3.344 \pm 0.440	3.457 \pm 0.275	3.327 \pm 0.339	3.592 \pm 0.250
Termination				
Liver weight (g)	16.807 \pm 2.197	15.764 \pm 3.609	16.800 \pm 2.459	14.916 \pm 1.916
Liver-to-body weight ratio	5.111 \pm 0.373	5.122 \pm 0.614	5.290 \pm 0.674	4.928 \pm 0.416
Females				
Day 11				
Liver weight (g)	0.748 \pm 0.092	0.742 \pm 0.071	0.707 \pm 0.113	0.707 \pm 0.291
Liver-to-body weight ratio	3.504 \pm 0.438	3.403 \pm 0.228	3.572 \pm 0.487	3.902 \pm 1.622
Termination				
Liver weight (g)	10.529 \pm 1.140	9.984 \pm 1.545	10.476 \pm 1.670	9.423 \pm 0.733
Liver-to-body weight ratio	4.887 \pm 0.340	4.964 \pm 0.535	5.031 \pm 0.541	4.959 \pm 0.400

^a Data obtained from pages 1230-1235 & 1242-1245, MRID 45441301.

N = 10/sex/dose

- c. **Clinical chemistry:** Serum T3 and T4 values were not affected by treatment on PND 11 or at termination. Table 16 summarizes these results.

Table 16. Thyroid T4 and T3 levels at day 11 and termination.

Dose Level	Day 11				Termination			
	T4		T3		T4		T3	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	3.65 \pm 0.68	4.14 \pm 0.94	0.56 \pm 0.07	0.61 \pm 0.08	5.33 \pm 0.67	3.63 \pm 1.28	0.94 \pm 0.19	0.99 \pm 0.11
100 ppm	4.02 \pm 0.61	4.45 \pm 0.48	0.56 \pm 0.10	0.61 \pm 0.17	5.60 \pm 0.99	3.92 \pm 0.78	0.85 \pm 0.08	0.94 \pm 0.19
500 ppm	4.12 \pm 0.80	3.98 \pm 0.69	0.55 \pm 0.10	0.58 \pm 0.12 (15%)	4.98 \pm 0.70	3.50 \pm 1.08	0.87 \pm 0.12	0.91 \pm 0.10
1000 ppm	3.77 \pm 0.52	4.19 \pm 0.54	0.48 \pm 0.05 (114%)	0.55 \pm 0.11 (110%)	5.12 \pm 0.98	3.55 \pm 0.95	0.91 \pm 0.10	0.96 \pm 0.15

Data from pages 1211 to 1219.

T3 levels for both males (14%) in the high dose group and females in the mid dose group (5%) and high dose group (10%) appear lower than the controls at day 11 only.

C. NEUROPATHOLOGY

- 1. Macroscopic examination:** No treatment-related effects were reported for male or female offspring at postnatal day 11 or study termination.
- 2. Microscopic examination:** No significant treatment-related effects were noted on postnatal days 11 or 70. Data on page 1183 for the 11 day old pups list only one animal with a abnormality in and this was a high dose male with "vacuolation" (grade 2). Data on page 1185 indicated that all examined thyroids were normal.

Data on pages 1187 to 1190 indicate only occasional abnormalities in the adult brain (6 levels), eye, dorsal root ganglion, gasserian ganglion, gastrocnemius muscle, sural nerve (left and right), sciatic nerve (left and right), tibial nerve (left and right), optic nerve, spinal cord (cauda equina and cervical). Axonal degeneration was noted in 6 control males and only one high dose male and one in each of the control and high dose females. There was no increase in degeneration severity score. Adult thyroid data (page 1192) indicated only a control female with cystic follicles). Selected data are presented in Table 17.

TABLE 17. Selected Histopathology Data (incidence/#examined) ^a		
Parameter	Dose (ppm)	
	0	1000
Males		
Day 11		
Brain- Level 6 Vacuolization	0/10	1/10 (2.0)
Termination		
Left Sural Nerve Axonal Degeneration	2/10 (1.0)	0/10
Left Tibial Nerve Axonal Degeneration	1/9 (1.0)	0/10
Right Tibial Nerve Axonal Degeneration	1/10 (1.0)	1/10 (1.0)
Lumbar Spinal Cord Axonal Degeneration	1/10 (1.0)	1/10 (1.0)
Thoracic Spinal Cord Axonal Degeneration	1/10 (1.0)	0/10
Spinal Nerve Root Axonal Degeneration	0/10	1/10 (1.0)
Females		
Termination		
Brain- Level 5 Vacuolization	0/10	1/10 (2.0)
Eye dysplasia	1/10 (2.0)	0/10
Cervical Spinal Cord Axonal Degeneration	1/10 (1.0)	1/10 (1.0)
Thoracic Spinal Cord Axonal Degeneration	0/10	1/10 (1.0)
Thyroid Cystic Follicles	1/10 (2.0)	0/10

^a Data obtained from pages 1183-1192, MRID 45441301.

N = 10/sex/dose. Number in parentheses is average severity of lesions: 1 (minimal) to 5 (severe).

Brain morphometry: Data are summarized in Table 18. There was a statistically significant ($p \leq 0.01$) decrease (25%) in the corpus callosum of male pups at the high dose on PND 11. No other treatment-related morphometric effects were observed in any animals on PND 11 or at study termination. Because of the concern for this effect, brain morphometric data from the low and mid dose groups are requested.

MKH 3586 (Amicarbazone)/114004

TABLE 18. Mean (\pm SD) morphometric data in offspring^a

Parameter	Dose (ppm)			
	0	100	500	1000
Males				
Day 11				
Anterior to posterior cerebrum length (mm)	12.46 \pm 0.37	12.17 \pm 0.36	12.64 \pm 0.35	12.03 \pm 0.65
Anterior to posterior cerebellum length (mm)	7.86 \pm 0.30	7.66 \pm 0.34	7.41 \pm 0.48	7.42 \pm 0.65
Frontal cortex thickness (mm)	1.697 \pm 0.014	NA	NA	1.521 \pm 0.014
Parietal cortex thickness (mm)	1.664 \pm 0.006	NA	NA	1.632 \pm 0.006
Caudate putamen (mm)	2.806 \pm 0.101	NA	NA	2.889 \pm 0.156
Corpus Callosum (mm)	0.631 \pm 0.015	NA	NA	0.473 \pm 0.017* (125%)
Hippocampal gyrus (mm)	1.245 \pm 0.007	NA	NA	1.237 \pm 0.011
External germinal (mm)	0.100 \pm 0.0002	NA	NA	0.095 \pm 0.0003
Cerebellum- roof to dorsal (mm)	3.916 \pm 0.167	NA	NA	3.981 \pm 0.207
Termination				
Anterior to posterior cerebrum length (mm)	14.75 \pm 0.35	14.83 \pm 0.31	14.75 \pm 0.34	14.81 \pm 0.40
Anterior to posterior cerebellum length (mm)	7.69 \pm 0.63	7.72 \pm 0.64	7.81 \pm 0.78	7.61 \pm 0.67
Frontal cortex thickness (mm)	1.921 \pm 0.004	NA	NA	1.923 \pm 0.007
Parietal cortex thickness (mm)	2.062 \pm 0.007	NA	NA	2.035 \pm 0.003
Caudate putamen (mm)	3.763 \pm 0.014	NA	NA	3.591 \pm 0.005
Corpus Callosum (mm)	0.441 \pm 0.009	NA	NA	0.442 \pm 0.003
Hippocampal gyrus (mm)	1.855 \pm 0.042	NA	NA	1.846 \pm 0.019
Cerebellum- roof to dorsal (mm)	5.774 \pm 0.144	NA	NA	5.595 \pm 0.077
Females				
Day 11				
Anterior to posterior cerebrum length (mm)	12.15 \pm 0.35	12.32 \pm 0.36	12.29 \pm 0.55	11.91 \pm 0.72
Anterior to posterior cerebellum length (mm)	7.48 \pm 0.34	7.49 \pm 0.41	7.52 \pm 0.35	7.39 \pm 0.14
Frontal cortex thickness (mm)	1.569 \pm 0.038	NA	NA	1.509 \pm 0.033
Parietal cortex thickness (mm)	1.624 \pm 0.008	NA	NA	1.605 \pm 0.009
Caudate putamen (mm)	2.673 \pm 0.163	NA	NA	2.786 \pm 0.099
Corpus Callosum (mm)	0.602 \pm 0.029	NA	NA	0.542 \pm 0.026
Hippocampal gyrus (mm)	1.187 \pm 0.005	NA	NA	1.177 \pm 0.018
External germinal (mm)	0.094 \pm 0.0001	NA	NA	0.088 \pm 0.0001
Cerebellum- roof to dorsal (mm)	4.023 \pm 0.082	NA	NA	3.963 \pm 0.162
Termination				
Anterior to posterior cerebrum length (mm)	14.44 \pm 0.29	14.23 \pm 0.45	14.23 \pm 0.29	14.15 \pm 0.44
Anterior to posterior cerebellum length (mm)	7.71 \pm 0.78	7.57 \pm 0.75	7.59 \pm 0.54	7.63 \pm 0.65
Frontal cortex thickness (mm)	1.862 \pm 0.005	NA	NA	1.834 \pm 0.016
Parietal cortex thickness (mm)	1.947 \pm 0.006	NA	NA	1.921 \pm 0.003
Caudate putamen (mm)	3.617 \pm 0.013	NA	NA	3.532 \pm 0.009
Corpus Callosum (mm)	0.469 \pm 0.008	NA	NA	0.395 \pm 0.004
Hippocampal gyrus (mm)	1.749 \pm 0.013	NA	NA	1.759 \pm 0.007
Cerebellum- roof to dorsal (mm)	5.491 \pm 0.042	NA	NA	5.292 \pm 0.060

^a Data obtained from pages 1161-1162, 1167-1168, 1173-1176, & 1178-1181, MRID 45441301.

N = 10/sex/dose

(%) decrease compared to controls and statistical analysis conducted by reviewer.

III. DISCUSSION AND CONCLUSIONS:

- A. INVESTIGATORS' CONCLUSIONS:** The investigators concluded that the overall NOEL is 100 ppm for dams and offspring based on decreased body weight. There were no morphologic changes in neural tissues and no adverse thyroid hormone levels in offspring.
- B. REVIEWER COMMENTS:** Treatment-related effects were limited to body weight and gain, increased food consumption and decreased food efficiency. During *gestation*, body weight at 1000 ppm was decreased ($p < 0.05$) of 5% on GD 6, with differences persisting to GD 20 (4%, $p < 0.05$) associated with a 12% decrease ($p < 0.05$) in body weight gain from GD 0 to GD 20. The high dose group was also associated with increased food consumption (13%) and associated decrease in feed efficiency (22%). During *lactation*, maternal body weight was decreased for high-dose rats; decreases averaged 4% on LD 0 to 9% on LD 14 and LD 21 ($p < 0.05$ or 0.01). Non significantly lower body weights were noted for the 500 ppm dose group of 2-3% but body weight gain during lactation was reduced 28% at 500 ppm and 45% at 1000 ppm. Food consumption during lactation was increased 7% at 500 ppm and 8% at 1000 ppm. The combination of body weight decrease and increase food consumption resulted in a decrease in food efficiency or 33% at 500 ppm and 49% at 1000 ppm.

The maternal LOAEL is 500 ppm (39 mg/kg/day) based primarily on decreased feed efficiency (combination of decreased body weight gain and increased feed consumption) during lactation. The maternal NOAEL is 100 ppm (8 mg/kg/day).

Treatment had no adverse effects on offspring survival, food consumption, clinical signs, developmental landmarks, FOB, motor activity, auditory startle reflex, learning and memory or brain weight. Decreased body weight and body weight gain were seen in males and females at the mid (500 ppm) and high (1000 ppm) dose groups. At birth, the average body weight of treated offspring was not different from controls at any dose level. By PND 11, body weight was decreased 7-8% ($p < 0.05$) for high-dose male and females, this decrease averaged 11-12% ($p < 0.01$) by weaning on PND 21. Body weight gain was decreased in males and females at the mid (8-13 %; $p < 0.05$ or $p < 0.01$) and high (11-21 %; $p < 0.05$ or $p < 0.01$) dose groups. Offspring in the 500 ppm group had recovered by termination; however, decreased body weight gain persisted to study termination in high-dose animals. On PND 11, the absolute brain weights were decreased in males (12%) and females (7%) at the high dose when compared to controls. On PND 11, there was a statistically significant ($p \leq 0.01$) decrease (25%) in corpus callosum of males at the high dose when compared to controls.

The offspring LOAEL is 1000 ppm (91 mg/kg/day) based on decreased body weight gain, decreased absolute brain weight, and brain morphometric changes. The offspring NOAEL is 500 ppm (39 mg/kg/day).

This study is classified **Acceptable/Non Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) pending receipt and review of the brain morphometric data for the low and mid dose groups, and review of the of positive control data.

C. STUDY DEFICIENCIES:

- Lack of statistical evaluation of the corpus callosum data in males when there was a 25% decrease and the lack of evaluation of this parameter at the low and mid dose groups as recommended by the Guideline.



13544



R117766

Chemical: Amicarbazone

PC Code:
114004

HED File Code: 13000 Tox Reviews

Memo Date: 7/27/2005

File ID:

Accession #: 412-06-0009

HED Records Reference Center
2/17/2006

